

Comparative Studies of Certain Cultures of *Puccinia rubigo-vera* and *Puccinia tomipara* on Wild Grasses

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COMPARATIVE STUDIES OF CERTAIN CULTURES OF PUCCINIA
RUBIGO-VERA AND PUCCINIA TOMIPARA
ON WILD GRASSES¹

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INTRODUCTION

The leaf rust of grasses, *Puccinia rubigo-vera* (DC.) Wint. probably is the most composite rust species known from the standpoint of the phenomena of host specialization. Mains (24) distinguished 56 races within this species, one specialized to rye, one to wheat, and 54 to combinations of wild-grass hosts. Physiologic specialization has been most extensively studied in the races on the cereals. Within the rye race, *Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton (*Puccinia dispersa* Eriks.) two physiologic forms have thus far been differentiated by Mains (22). Within the wheat race, *Puccinia rubigo-vera* sp.f. *tritici* (Eriks.) Carleton (*Puccinia triticina* Eriks.) at least 53 physiologic forms are known to exist. These are distinguished by their reaction to a differential set of agronomic varieties of wheat, (25), (15).

In the wild grasses there has not been such a selection and differentiation of varieties as has occurred in cultivated grasses. However, this does not exclude the possibility of the existence within wild-grass species of strains that differ in their reaction to rust.

It was the purpose of the investigations here presented to make a study of several cultures of *Puccinia rubigo-vera*, and of the wild grasses that serve as their hosts, in an effort to obtain some information concerning the extent to which the physiologic specialization of these cultures is comparable to the specialization of the leaf rust of cereals.

¹ Papers from the Department of Botany and the Herbarium of the University of Michigan no. 504.

² The writer very gratefully acknowledges the helpful criticism and advice accorded him by Dr. E. B. Mains, under whose supervision these investigations were conducted. He is also grateful to the personnel of the Botanical Gardens of the University of Michigan for the facilities placed at his disposal and for the many courtesies shown.

HISTORICAL SKETCH

The history of *Puccinia rubigo-vera* began in 1815, when de Candolle (6) gave the name *Uredo rubigo-vera* to a rust on wheat, in France. Winter (36) in his treatment of the rusts in Rabenhorst's "Kryptogamen-Flora," assigned this species to the genus *Puccinia* and recognized that it occurred on a number of grasses. With the development of knowledge concerning host restriction, especially with regard to aecial host, various groups have been segregated as species, races, forms, etc.

Thirty-five botanists have proposed 65 names for segregates of *Puccinia rubigo-vera*. Arthur and Fromme (4) have distributed these under 5 rusts: *Dicaeoma Asperifolii* (Pers.) Kuntze, *D. apocryptum* (Ellis and Tracy) Kuntze, *D. procerum* (Dietel and Holway) Kuntze, *D. Cockerellianum* (Bethel) Arthur and Fromme, and *D. Clematidis* (DC.) Arthur. These species differ chiefly in having aecial hosts belonging to different families of plants. Recently, however, Mains (24) united these species and recognized a number of more or less sharply differentiated races, in the resulting composite species, *Puccinia rubigo-vera*. These races were named by Mains (*op. cit.*) and separated by virtue of aecial and telial host combinations and grass host ranges. According to this concept of *Puccinia rubigo-vera*, 86 species of plants, representing 25 genera, and 4 families, serve as aecial hosts for the 56 known physiologic races of this rust species. More than 90 species in 16 genera of grasses serve as uredial and telial hosts. These are distributed in 4 tribes, *viz.*, Agrostideae, 2 races; Avenae, 3 races; Festuceae, 14 races; and Hordeae, 38 races. Still more recently, Arthur (5), in his "Manual of the Rusts in United States and Canada," has recognized this composite species, *Puccinia rubigo-vera*, but divides it into 6 varieties on the basis of aecial and telial host combinations, aided in some cases by spore size and geographic distribution. A complete history of *Puccinia rubigo-vera*, and an organization of the synonymy have been given by Mains (24).

The race restricted to wheat, *Puccinia rubigo-vera*, sp.f. *tritici* (Eriks.) Carleton, has been studied most extensively, especially with regard to physiologic specialization. Mains and Jackson (25) were able to distinguish 12 physiologic forms of the leaf rust of wheat in the United States by the differential reaction of 11 agronomic varieties. Since then, contributions by Scheibe (27) (28), Waterhouse (33), Johnston (14), Johnston and Mains (15), and others have brought the number of known forms up to 53. That some of these forms can be further divided into sub-forms by the use of additional differential wheat varieties has been shown by Waterhouse (33) (34), and Scheibe (28).

No wild-grass race of *Puccinia rubigo-vera* has been studied so inten-

sively; as a result, comparatively little is known regarding host specialization within the wild-grass races.

A few earlier investigators, working with cultures of wild-grass races of *Puccinia rubigo-vera* have noticed differences in reaction between different collections of wild-grass species.

Thus Mains (24, P. 384) found that,

one collection of *Hordeum jubatum* apparently was resistant to culture Th. 15 of the race *vulgaris* (Table II) and to race *occidentalis* (Table III) and susceptible to other cultures of these races. Likewise one collection of *Elymus virginicus* was apparently resistant to one culture of race *anomala* (Table II) and to one of race *Impatiens* (Table XIII), though susceptible to the other cultures of these races. Though these instances are hardly sufficient to justify conclusions, they suggest that some at least of the wild-grass races may be subdivided through differences in reaction of selections or strains of the various species of grasses.

Mains (*op. cit.* P. 384) further asserts,

There is no reason to assume that the races with wild-grass hosts differ in this respect from the race *Tritici* on wheat. The last has received extensive study in this regard, but too few collections of both the wild-grass races and their hosts have been studied to justify a comparison.

Mains (23) noted strain differences in resistance and susceptibility in the hosts of *Puccinia dispersa* Eriks. (*Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton), *P. agropyrina* Eriks. (*P. rubigo-vera* sp.f. *persistens* (Plowright) Mains), *P. montanensis* Ellis, and *P. andropogonis* Schw.

Hassebrauk (12), obtained some very interesting results in his studies of the reactions of collections of numerous wild grasses to the several cereal rusts. Besides finding that in many cases the wild grasses are susceptible to the particular forms of cereal rusts that he used, he observed many cases in which a number of collections of the same grass species from different sources behaved quite differently as to rust reaction. For instance, one of his two collections of *Elymus virginicus* was fairly susceptible to form 14 of *Puccinia triticina* Eriks. (*P. rubigo-vera* sp.f. *tritici*), whereas the other was resistant. The two collections had come from widely separated sources.

In this investigation it was thought best to employ a few races of *Puccinia rubigo-vera* and a number of species of grasses. Several collections of each host species, usually from different localities, were obtained in order to study the possibility of reactional differences within grass species.

The investigations of *Puccinia tomipara* are a natural outgrowth of the studies of *P. rubigo-vera* since the former has been considered a race of the latter (3). It differs from *P. rubigo-vera* in producing multicellular telio-

spores. *P. tomipara* was described by Trelease (32) who found it infecting *Bromus ciliatus* L. Arthur (1) considered this to be merely a morphologic variation sometimes met in grass rusts, and when he (1) connected aecia on *Clematis virginiana* L. with telia of the leaf rust type on *Bromus ciliatus*, having normal two-cell teliospores, he therefore used the name *P. tomipara* for this rust. He also used this name again (2) in designating a successful infection of *C. virginiana* from two-cell teliospores from *B. ciliatus*. It is to be noted that these *Clematis*-*Bromus* connections were really obtained with typical collections of *P. rubigo-vera*.

Fraser (10) was the first to establish the true aecial connection of *Puccinia tomipara*. Field evidence suggested the connection of aecia on *Thalictrum dasycarpum* Fisch. and All. with leaf rust on *Bromus ciliatus* and species of *Elymus* and *Agropyron*. When he inoculated these grasses with aeciospores from *T. dasycarpum*, heavy infection resulted on *Elymus canadensis* L., *E. virginicus* L. and *B. ciliatus* with uredia and telia in great abundance. Microscopic examination of the telia ultimately resulting from these inoculations showed those on the *Elymus* spp. to be of the normal two-cell leaf rust type. The teliospores taken from *B. ciliatus*, however, were

very variable in size, shape and number of cells, only rarely could two-celled spores of the *Puccinia* type be found, practically all of the spores being three to several celled, some having as many as sixteen cells.

Fraser (10) inoculated *Elymus virginicus*, *Agropyron tenerum* Vasey, *A. Smithii* Rydb., *A. repens* (L.) Beauv. and *Hordeum jubatum* L. with this *Bromus* rust but no infection resulted. This rust was clearly *Puccinia tomipara*. Fraser stated that the multicellular character seemed fixed, inasmuch as many collections, both on *Bromus ciliatus* and *B. latiglumis* (Shear) Hitch. (*B. altissimus* Pursh.), showed this character. He considered it best to include the rust under *P. agropyri* E. and E. until further study established its true position. Fraser also stated that it seemed doubtful whether the rust should even be included in the genus *Puccinia*.

Arthur *et al* (3) considered *Puccinia tomipara* to be a teratologic race, into which category they also put the related species, *P. elymi* Westend., a multicellular form on *Elymus*, in which the cells of the teliospores are arranged in linear fashion.

Lagerheim (18) erected the genus *Rostrupia* on the strength of Westendorp's (35) *Puccinia elymi* and Trelease's *P. tomipara*, considering the multicellular character of the teliospores of these rusts to be a good generic character. Since then several rusts have been assigned to this genus by Dietel (7), but Arthur *et al*. (4), in the North American Flora, reduced these species to synonymy insofar as they apply to North American rusts. Accordingly, *P. elymi* and *P. tomipara* are listed by Arthur *et al*. (4) in the

synonymy for *Dicaeoma clematidis* (DC.) Arth. Mains (24), on the other hand, did not include these two grass rusts in *P. rubigo-vera*, and stated that it seemed best, for the time being, to regard them as distinct species until culture work has shown whether the multicellular character is or is not stable. More recently, Arthur (5) in his "Manual of the Rusts in United States and Canada," cites *P. tomipara* and *P. elymi* as synonyms of *P. rubigo-vera*.

MATERIALS AND METHODS

In these studies 111 collections of species and varieties of wild grasses were used; 65 belong to the genus *Bromus*; 46 belong to the tribe *Hordeae*, including 20 numbers of 9 species and varieties of *Elymus*, 7 numbers of 4 species of *Agropyron*, 17 numbers of 5 species of *Hordeum* and 1 number of *Hystrix patula* Moench. It will be noted that in many instances the various species are represented by more than a single collection.³

Although 20 collections of *Puccinia rubigo-vera* were studied to some extent, only 9 of these received concentrated study and are reported here. One culture of *P. tomipara* also is included. These rust cultures are referred to in the following pages by numbers only.

The aecial hosts of most of the cultures were determined as a result of clues obtained from field associations. Thus cultures 1, 13, and 20 were connected with *Thalictrum*; 14, 18, 21 with *Impatiens*; 22 and 23 with *Clematis*; connections of cultures 8 and 11 were not determined. Herbarium specimens of the various cultures have been deposited in the herbarium of the University of Michigan.

In Table 1 is given a summary of all the field data concerning these cultures of *Puccinia rubigo-vera* and of *P. tomipara*.

The inoculation methods used in the present investigations have been described by Mains (21). Minor modifications are explained below.

On the most susceptible grass for each rust culture, attempts were made to build up a supply of inoculum with which to inoculate a series of grasses. If these "stock cultures" were watered from below for a few days prior to use for inoculation, the urediospores piled up on each uredium, resulting in the accumulation of a considerable supply of inoculum.

In the process of inoculation, in addition to shaking the heavily rusted "stock plants" over the previously atomized grasses, it was found advan-

³ The numbers appended to the grass collections are the accession numbers assigned to them by Dr. E. B. Mains who was instrumental in obtaining most of them, especially from foreign botanical gardens. Numbers preceded by B. G. are accession numbers of the Botanical Garden of the University of Michigan. Acknowledgement and thanks are due Dr. J. R. Swallen, of the Smithsonian Institution, and to Prof. J. H. Ehlers of the Department of Botany of the University of Michigan, for checking the identification of the grasses used.

TABLE 1.—*Cultures of Puccinia rubigo-vera obtained from Michigan, Indiana, Ohio and Kansas and of P. tomipara from Michigan*

Culture	Hosts in Field		Where Collected	Collector
	Aecial	Telial		
<i>Puccinia rubigo-vera</i>				
1	<i>Thalictrum</i> ¹ spp.	<i>Agropyron repens</i>	Ann Arbor, Mich.	G. W. Fischer
8	undetermined	<i>Agropyron repens</i>	Angola, Ind.	G. W. Fischer
11	undetermined	<i>Elymus virginicus</i>	Manhattan, Kansas	C. O. Johnston
13	<i>Thalictrum dioicum</i>	<i>Agropyron tenerum</i>	Pinckney, Mich.	G. W. Fischer
14	<i>Impatiens biflora</i>	<i>Elymus virginicus</i>	Ann Arbor, Mich.	G. W. Fischer
18	<i>Impatiens biflora</i>	<i>Elymus virginicus</i>	Adam's Mills Ohio	E. B. Mains
21	<i>Impatiens biflora</i>	<i>Elymus virginicus</i> <i>Elymus striatus</i>	Ann Arbor, Mich.	G. W. Fischer
22	<i>Clematis virginiana</i>	<i>Elymus virginicus</i>	Marquette, Mich.	E. B. Mains
23	<i>Clematis virginiana</i>	<i>Bromus ciliatus</i> <i>Bromus altissimus</i>	Pinckney, Mich.	G. W. Fischer
<i>Puccinia tomipara</i>				
20	<i>Thalictrum dasy-</i> <i>carpum, T. dioicum</i>	<i>Bromus ciliatus</i> <i>B. altissimus</i>	Ann Arbor, Mich.	G. W. Fischer

¹ Determined by inoculation experiments only

tageous to draw these back and forth over the wet grasses very much after the manner in which one would use a paint brush. A more satisfactory distribution of inoculum was effected in this manner than by any other method tried. After inoculation the whole was then incubated 12 to 24 hours under 2 or 3 layers of wet muslin. When uncovered, the grasses were always wet with condensed moisture, thus simulating dew in the field.

Uredia usually began to appear in 8 to 10 days on the most susceptible grasses, but data were not taken until at least 2 weeks after inoculation in order to allow ample time for full expression of reaction. A card file of the grasses proved to be convenient when taking data.

In order to avoid contaminations of one rust culture with another, each was kept, together with its individual series of grasses, in a separate compartment of a portion of the greenhouses especially constructed for rust and mildew investigations at the Botanical Gardens of the University of Michigan.

For inoculation purposes seedlings of the grasses were used, for, in

this stage of growth, the hosts would naturally be in a vigorous condition and a more reliable rust reaction would be secured. Grasses to be inoculated were grown in 2½-inch pots, with 5 to 15 seedlings in each, and were ready for inoculation after 6 or 7 weeks. It is recognized that, in some cases at least, susceptibility reported here for grasses in the seedling stage does not necessarily imply a similar susceptibility in the field or when mature. It has been noted by previous investigators of grass and cereal rusts, notably Johnston and Melchers (16), and Stakman and Piemeisel (30), that certain varieties or strains of the host plant might show a high degree of susceptibility in the seedling stage, but be highly resistant at heading time.

Although it was often found that the types of reaction of the wild grasses to leaf rust are not so clearly defined as are those to the leaf rusts of cereals (a fact also reported by Mains (24)), the symbols used in designating such reactions on cereals have, nevertheless, been found convenient. They are those employed by Stakman and Levine (29) for stem rust, *Puccinia graminis*, and by Mains and Jackson (25) in their studies on the leaf rust of wheat, modified slightly for adaptation to the present studies as follows:

- | | |
|---------------------------------|--|
| 0. Highly resistant | No uredia produced; various degrees of flecking produced in some cases, entirely absent in others. |
| 1. Very resistant | Uredia few, very small, often abortive; in some cases accompanied by pronounced necrosis; necrotic spots often without uredia. |
| 2. Moderately resistant | Uredia better developed than in the preceding; necrotic spots present but less pronounced and not without uredia. |
| 3. Moderately susceptible | Uredia well developed, of moderate size; never accompanied by necrotic areas. |
| 4. Very susceptible | Uredia relatively large, well-developed, and capable of copious production of urediospores. |

The method used in inoculating aecial hosts is the standard one whereby the overwintered telia, under the proper conditions of temperature and moisture, are suspended over the plants to be inoculated. For the most part the plant cover recommended by Mains (21), consisting of a wooden "house" with a glass "roof," was used. The telia of the rust cultures were overwintered in loose cheese-cloth bags or loosely wrapped in mosquito netting. In the spring, as soon as the teliospores showed sufficient germination, inoculations of the aecial host were begun.

RESULTS

Cultural Experiments with *Puccinia rubigo-vera*

Culture 1. On Agropyron repens and Thalicttrum spp.—This culture was collected on couch or quack grass, *Agropyron repens*, at the Botanical

Garden of the University of Michigan, and represents a leaf rust that is very prevalent throughout the lower peninsula of Michigan.

Culture 1 was especially adapted to greenhouse study. It was easily propagated in the uredial stage on either seedlings of quack grass or on plants grown from potted rhizomes from the field. Unlike most of the other rust cultures studied, culture 1 produced telia in very scant quantities.

This culture of leaf rust of *Agropyron repens* is very like that found in England on the same host and described by Plowright (26) as *Puccinia persistens*, or the rust designated by Eriksson and Henning (9) as *P. dispersa* f. *agropyri*, and that Eriksson (8) later raised to specific rank as *P. agropyrina*. Mains (24) considered that these names all refer to one race of *P. rubigo-vera*. This he designated as *P. rubigo-vera* sp.f. *persistens* (Plowright) Mains.

Inoculations of a series of grasses during the past two years have given the following results:

DISTINCTLY SUSCEPTIBLE (reaction type 3-4): *Agropyron dasy-stachyum* (Hook.) Scrib. (1656), *A. repens* (L.) Beauv. (1467, B.G. 13007), *A. tenerum* Vasey (1484, 1659), *Elymus glaucus* Buckley (1539), *Hordeum gussoneanum* Parl. (817, 1393, 1421, 1457), *H. jubatum* L. (1647), *H. murinum* L. (1548).

MODERATELY SUSCEPTIBLE (reaction type 2-3): *Elymus canadensis* L. (1640), *E. virginicus* L. (1793), *Hordeum gussoneanum* (1291, 1433), *H. murinum* (1510), *H. pusillum* Nutt. (1650).

DISTINCTLY RESISTANT (reaction type 0-2): *Agropyron caninum* (L.) Beauv. (1466, B.G. 12770, 803), *Elymus glaucus* (1482), *E. robustus* (S. and S.) Mack and Bush (1642), *E. striatus* Willd. (1645), *E. striatus arkansanus* (S. and B.) (Hitchc. (780)), *E. virginicus* (765, 842, 1475, 1476, 1538), *E. virginicus glabriflorus* (Vasey) Bach. (1646), *E. virginicus submuticus* Hook. (793), *Hordeum murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* L. (1509), *Hystrix patula* (1477).

Plowright (26) showed that the leaf rust of *Agropyron repens* in England produces its aecia on *Thalictrum flavum* L. and possibly *T. minus* L. Treboux (31) reported having connected aecia on *Clematis Pseudo-flammula* with uredia and telia on *A. repens*. Mains (24) reported aecia having been produced on *T. delavayi* Franchet, *T. flavum* and *T. minus* from telia of the leaf rust of *A. repens*.

In the present experiments, overwintered telia of *Puccinia rubigo-vera* on *Agropyron repens* suspended over species of *Thalictrum* in an inoculating chamber, resulted in the production of pycnia and aecia on *Thalictrum minus*, *T. glaucum*, *T. flavum* and *T. fendleri*. No infection resulted on *T. dioicum* L., and *T. polygamum*. Similar inoculations of *Clematis virginiana* and *Impatiens biflora* Walt. yielded negative results.

Jackson and Mains (13) pointed out the evident relationship of the leaf rust of *Agropyron repens* to the leaf rust of wheat, emphasizing the fact that they have similar aecial hosts and are morphologically similar. The cultural behavior of the former in the greenhouse is strikingly like that of the latter.

Culture 8. On Agropyron repens (Aecial Host Undetermined). This culture obviously is related to Culture 1, but is physiologically distinct. It was collected at Angola, Indiana, on *Agropyron repens*. No telia were present, and since there was only a very scant production of these sori in the greenhouse, no attempt was made to determine aecial-host connections.

Inoculations of a series of grasses with culture 8 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron repens* (1467), *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482, 1539), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Agropyron tenerum* (1489), *Elymus virginicus* (1475), *Hordeum murinum* (1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770, 1466), *A. repens* (B.G. 13007), *Elymus canadensis* (843), *E. striatus arkansanus* (780), *E. virginicus* (842, 1476, 1538), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (1292), *H. murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* (1435), *Hystrix patula* (1477).

It will be seen from the above data that cultures 8 and 1 differ chiefly in the marked susceptibility to the former of certain collections of *Elymus canadensis*.

Culture 11. On Elymus virginicus (Aecial Connection Undetermined). This culture came from Manhattan, Kansas, where C. O. Johnston collected it on *Elymus virginicus*. It was easily and readily multiplied on plants of *E. virginicus* in any stage of development, so that an abundant supply of inoculum was produced. Telia were rarely produced and then in such scant quantities that no attempt was made to determine the aecial host connections.

Inoculations of a series of grasses with culture 11 over a period of 2 years have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron dasystachyum* (1656), *A. tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366, 1640), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1476, 1793), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum murinum* (1548), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1482), *E. striatus arkansanus* (780), *E. virginicus* (1538).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. striatus* (1645), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510), *H. nodosum* (1435, 1509).

From these results it is seen that this culture of *Puccinia rubigo-vera* finds favorable hosts in a rather wide range of grasses. Eighteen collections of 8 species and 2 varieties, representing 4 genera, have proved to be distinctly susceptible.

Culture 13. On *Thalictrum dioicum* and *Agropyron tenerum*. On May 28, 1932, the writer found, near Pinckney, Michigan, plants of *Thalictrum dioicum* heavily infected with aecia. A considerable quantity of this material was collected and used to inoculate a series of grasses.

An abundance of plants of *Agropyron tenerum* and *Elymus virginicus*, heavily infected with telia of leaf rust, were collected in the same locality the following September. These were put outside to hibernate, but each host was kept separately.

In the spring of 1933, the overwintered telia on *Agropyron tenerum* were used to inoculate plants of *Impatiens biflora*, *Clematis virginiana*, *Thalictrum dioicum*, *T. dasycarpum*, *T. flavum*, and *T. glaucum*. Aecia were produced on the *Thalictrum* spp. only. Another set of the same plants were inoculated from the telia overwintered on *Elymus virginicus*; aecia resulted on *Impatiens biflora* only. This *Impatiens-Elymus* culture was not further studied.

Inoculations with this culture are not complete. However, the series of grasses inoculated gave the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366), *E. glaucus* (1482), *E. virginicus* (842, 1475), *Hordeum gussoneanum* (1433), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Elymus virginicus* (1476), *Hordeum gussoneanum* (1457), *Hystrix patula* (1477).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Hordeum gussoneanum* (817, 1421), *H. murinum* (743, 785, 1434), *H. nodosum* (1435).

Apparently this is the first demonstration of the connection of *Puccinia rubigo-vera* on *Agropyron tenerum* with *Thalictrum* spp. either by culture experiments or field observations.

Culture 14. On *Elymus virginicus* and *Impatiens biflora*. This culture of leaf rust originated from a collection made by the writer on *Elymus virginicus*, northwest of Ann Arbor, in September, 1931. Uredia and telia were present, the latter in abundance; both were collected. The uredio-

spores were sown on seedlings of *Elymus virginicus* in the greenhouse, giving rise to uredia in 9 to 10 days. The telia were not successfully overwintered. In the fall of 1932 more telia were collected from the same region. These were overwintered successfully, and good germination was obtained the following spring. This collection was used to inoculate *Clematis virginiana*, *Thalictrum dioicum*, and *Impatiens biflora*; pycnia and aecia were produced on the last, only.

The greenhouse culture of the rust on *Elymus virginicus* seedlings was multiplied with fair success for a few weeks. However, during the months of November, December, and January, the uredial culture was nearly lost, the uredia being gradually supplanted by telia. It was not until after February that this culture could be multiplied in the uredial stage.

Repeated inoculations with culture 14 of *Puccinia rubigo-vera* yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. dasystachyum* *E. robustus* (1642), *E. striatus arkansanus* (780), *E. virginicus* (765, 842, (1656), *A. tenerum* (1484), *Elymus canadensis* (795, 1305, 1366, 1640), 1475, 1793), *E. virginicus submuticus* (793), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1539), and *E. virginicus* (1476).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. tenerum* (1659), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (1538), *E. virginicus glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1548), *H. nodosum* (1435, 1509), *H. pusillum* (1650).

Culture 18. On Elymus virginicus and Impatiens biflora. This culture of leaf rust came from a collection of telia on *Elymus virginicus* made by E. B. Mains, at Adams Mills, Ohio, September, 1931. These telia were overwintered in the usual manner and a good germination of the teliospores resulted. These were used to inoculate potted plants of *Impatiens biflora*, *Thalictrum fendleri*, *T. dasycarpum*, *T. flavum*, *T. glaucum*, *Ranunculus acer*, *Anemone virginiana* and *Clematis virginiana*. Pycnia and aecia developed on *I. biflora*, only, from which infection the aeciospores were sown on seedlings of *E. virginicus* (842, 1475). A vigorous development of uredia resulted. The culture was multiplied and the resulting inoculum oversummed at a temperature of 10° to 12° C. The following October the culture was renewed from these stored urediospores. A fair infection on *Elymus virginicus* resulted. The uredia gradually reverted to telia until, by the middle of December, the uredial culture was lost entirely. Abundant telia, however, were produced and overwintered and the culture was renewed the

following spring (1932) from inoculations on *Impatiens biflora*. Grass inoculations were carried out in early summer.

Inoculations of a series of grasses with culture 18 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1659), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1539), *E. striatus* (1645), *E. virginicus* (765, 842, 1475, 1476, 1538, 1793), *Hordeum nodosum* (1509).

MODERATELY SUSCEPTIBLE: *Agropyron caninum* (1466), *Elymus robustus* (1642), *E. virginicus glabriflorus* (1646), *Hordeum jubatum* (1647).

DISTINCTLY RESISTANT: *Agropyron caninum* (B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus canadensis* (1640), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. pusillum* (1650).

Culture 21. On Elymus striatus and Impatiens biflora. Culture 21 of leaf rust came from a collection of uredia and telia on *Elymus striatus* that the writer made 5 miles south of Ann Arbor in October, 1932. The uredia were used to start the culture on plants of *E. striatus* brought back from the locality where the rust was collected. The telia were put outside to overwinter. They germinated well the following spring and were used to inoculate plants of *Impatiens biflora*, *Clematis virginiana*, *Thalictrum dioicum*, *T. dasycarpum*, *T. glaucum*, *T. flavum*, and *T. minus*. Pycnia and aecia were produced on *I. biflora*, only. The aeciospores on *Impatiens* were used to start the culture from which the grass inoculations were made.

Inoculations of a series of grasses with culture 21 yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Elymus canadensis* (795, 843), *E. robustus* (1642), *E. virginicus* (842, 1476, 1793), *E. virginicus* var. *submuticus* (793).

MODERATELY SUSCEPTIBLE: *Elymus canadensis* (1537, 1640), *E. glaucus* (1539), *E. striatus* var. *arkansanus* (780), *E. virginicus* (1475), *Hordeum gussoneanum* (1421), *H. pusillum* (1650).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. dasystachyum* (1656), *A. repens* (1467, B.G. 13007), *A. tenerum* (1659), *Elymus australis* (1644), *E. canadensis* (1305, 1366), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (765, 1538), *E. virginicus* var. *glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. nodosum* (1509), *Hystrix patula* (1477).

It is interesting to note that the *Elymus striatus* on which culture 21 was collected and later propagated was highly susceptible, while the *E.*

striatus (1645) from Indianapolis, Indiana, was distinctly resistant. Only moderate susceptibility was shown by the variety *arkansanus* (780) of *E. striatus*. Furthermore, culture 21 is the only culture of *Puccinia rubigo-vera* studied (culture 23 on *Bromus* spp., excepted) that had no susceptible hosts in the genera *Agropyron* and *Hordeum*.

Culture 22. On Clematis virginiana and Elymus virginicus. Culture 22 of *Puccinia rubigo-vera* was obtained from aecia collected on *Clematis virginiana* by E. B. Mains, at Marquette, Michigan, June, 1933. The aeciospores, sown on *Elymus virginicus* (1475) and *Bromus ciliatus* (1649), resulted in a heavy infection on *E. virginicus*, only. The culture was multiplied twice, and abundant inoculum was obtained to inoculate the usual series of grasses. Inoculation experiments were carried out during the summer.

Inoculations of the following grasses with culture 22 have given the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. tenerum* (1659), *Elymus canadensis* (1305, 1537), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1793), *Hordeum jubatum* (1647), *H. nodosum* (1509), *H. pusillum* (1650).

MODERATELY SUSCEPTIBLE: *Hordeum murinum* (1434, 1458, 1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. repens* (1467, B.G. 13007), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 843, 1366, 1640), *E. glaucus* (1482), *E. striatus* (1645), *E. striatus arkansanus* (780), *E. virginicus* (1476, 1538), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1548), *H. nodosum* (1435), *Hystrix pastula* (1477).

Table 2 summarizes the reactions of the 46 collections of species of *Agropyron*, *Elymus*, *Hordeum*, and *Hystrix* to the 8 cultures of *Puccinia rubigo-vera* described in the foregoing.

Culture 23. On Bromus ciliatus and Clematis virginiana. This culture was obtained accidentally while working with *Puccinia tomipara*. Overwintered telia on *Bromus ciliatus* collected near Pinckney, Michigan, in September, 1932, were being used to inoculate plants of *Thalictrum dioicum* and *T. dasycarpum* (April 29, 1933). A healthy potted plant of *Clematis virginiana* was also placed in the inoculation chamber along with the *Thalictrum* spp.

Pyenia appeared, not only on the *Thalictrum* spp., as expected, but also on *Clematis virginiana*, followed by aecia on both. Thus it appeared as if *Puccinia tomipara* produced aecia on *C. virginiana*, as well as on species of *Thalictrum*. The aeciospores from *C. virginiana* were used to inoculate

TABLE 2. Summary of the reactions of 46 collections of wild-grass species to 8 cultures of *Puccinia rubigo-vera*

Wild-grass collections	Cultures of <i>Puccinia rubigo-vera</i>							
	1	8	11	13	14	18	21	22
	TYPES OF REACTION							
<i>Agropyron caninum</i> 803	0	0	0	0	0	0	0	0
<i>A. caninum</i> 1466	0	0	0		3-4	3	2	3-4
<i>A. caninum</i> B.G. 12770	0	0	0	0	0	0	0	1-2
<i>A. dasystachyum</i> 1656	4		4		3-4	3-4	0	0
<i>A. repens</i> 1467	3-4	3-4	0	0	0	0	0	0
<i>A. repens</i> B.G. 13007	3-4	0	0	0	0	0	0	0
<i>A. tenerum</i> 1484	3-4	3	4	3-4	4			
<i>A. tenerum</i> 1659	3-4		3-4	4	0	3-4	0	3-4
<i>Elymus australis</i> 1644	0		0		0	3-4	0	0
<i>E. canadensis</i> 795	0	3-4	4	3-4	3-4		4	0
<i>E. canadensis</i> 843	0	0	0	0-1	0		3-4	0
<i>E. canadensis</i> 1305	0	3-4	4	3-4	3-4	4	0	4
<i>E. canadensis</i> 1366	0	3-4	3-4	3-4	3-4	3-4	0	0
<i>E. canadensis</i> 1537	0	3-4	0	0	0	3-4	3	3-4
<i>E. canadensis</i> 1640	2-3		3-4		3-4	2	3	0
<i>E. glaucus</i> 1482	0	4	3	4	0		0	0
<i>E. glaucus</i> 1539	3-4	4	4		3	3-4	3	3-4
<i>E. robustus</i> 1642	0		3-4		4	2-3	3-4	3-4
<i>E. striatus</i> 1645	0-2		0		0	3-4	0	0
<i>E. striatus</i> var. <i>arkansanus</i> 780	0	0-1	3		3-4	3-4	3	0
<i>E. virginicus</i> 765	0		3-4	2	3-4	3-4	0	4
<i>E. virginicus</i> 842	0	0	4	3-4	4	4	3-4	3-4
<i>E. virginicus</i> 1475	0	3	4	3-4	3-4	3-4	3	4
<i>E. virginicus</i> 1476	0	0	3-4	3	3	3-4	3-4	0-1
<i>E. virginicus</i> 1538	0	0	2-3	0	0	3-4	0	0
<i>E. virginicus</i> 1793	2-3		4		3-4	3-4	3-4	4
<i>E. virginicus</i> var. <i>glabriflorus</i> 1646	0		3-4		0	3	0	0
<i>E. virginicus</i> var. <i>submuticus</i> 793	0	0	3-4		3-4		3-4	0
<i>Hordeum gussoneanum</i> 817	3-4	4	0	0	0	0	0	0
<i>H. gussoneanum</i> 1291	2-3	0	0		0		0	0
<i>H. gussoneanum</i> 1393	3-4	3-4	0		0	0	1-2	1-2
<i>H. gussoneanum</i> 1421	3-4	3-4	0	0	0	0	3	1-2
<i>H. gussoneanum</i> 1433	2-3	3-4	0	3-4	0-1	0	0	0
<i>H. gussoneanum</i> 1457	3-4	4	0	3	0	0	0	2
<i>H. jubatum</i> 1647	4		0		0	2-3	0	3-4
<i>H. murinum</i> 743	0-2	0-1	0	0	0	0	0	0-2
<i>H. murinum</i> 785	0-2	0	0-2	0	0	0	0	2
<i>H. murinum</i> 1290	0-2	0	0-2		0	0	0	0-2
<i>H. murinum</i> 1434	0-2	0-2	0-2	0	0	0-1	0	2-3
<i>H. murinum</i> 1458	0-2	0	0-2		0		0	2-3
<i>H. murinum</i> 1510	2-3	3	0-2		0	0	0	2-3
<i>H. murinum</i> 1548	3-4	4	3-4	3-4	0	0-1	0	0
<i>H. nodosum</i> 1435		0	0	0	0			
<i>H. nodosum</i> 1509	0		0		0	3-4	0	3-4
<i>H. pusillum</i> 1650	3-4		0		0	2	3	3-4
<i>Hystrix patula</i> 1477	0-2	0	3-4	3	4		0-1	0

one pot each of *Bromus ciliatus* and *B. purgans*; two other pots of these grasses also were inoculated with aeciospores from *T. dioicum*. Uredia were abundant in both cases. Telia, however, did not appear, until early July

on the grasses inoculated with aeciospores from *T. dioicum*, and about 2 weeks later on the grasses inoculated with aeciospores from *C. virginiana*. Several mounts of the first telia contained the multicellular teliospores characteristic of *P. tomipara*; occasionally a two-cell spore could be found. The telia resulting from aeciospores from *C. virginiana*, however, were all bicellular; the culture clearly belonged to *P. rubigo-vera*, and was designated as culture 23.

It seemed desirable to compare the host range of this *Bromus* culture of *Puccinia rubigo-vera* with that of *P. tomipara*. Essentially the same series of *Bromus* species was inoculated with a uredial culture of *P. rubigo-vera* as was used for a similar study of *P. tomipara*.

Inoculations of a series of *Bromus* species have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus purgans* (1289), *B. ciliatus* (1649), *B. altissimus* (1795).

DISTINCTLY RESISTANT: *Bromus adoensis* Höchst. (1331, 815), *B. arduensis* Dum. (1332), *B. arvensis* L. (708, 1297), *B. brizaeformis* F. and M. (1442), *B. commutatus* Schrad. (716, 771), *B. hordeaceus* L. (719, 1508), *B. japonicus* Thumb. (1347), *B. kalmii* Gray. (1541), *B. macrostachys* Desf. (797, 714, 705, 1446), *B. madritensis* L. (713), *B. maximus* Desf. (1427), *B. rigidus* Roth. (1384, 1452, 1501, 698, 1505), *B. rigidus* var. *gussonii* (Parl.) C. and D. (1506, 1333), *B. rubens* L. (1502), *B. sterilis* L. (701, 1503), *B. squarrosus* L. (1450), *B. tectorum* L. (1547, 768, 1545, 712), *B. uniloides* (Willd.) H.B.K. (1507, 1451, 718, 750).

Comparative Morphology of the Urediospores and Teliospores of Eight Cultures of Puccinia rubigo-vera. Attempts to discover morphological differences between races or between physiologic forms within composite rust species have been made by few investigators. Levine (19) has shown that there are significant morphological differences between the spore forms of the 5 races of *Puccinia graminis* that he studied and that these races can, therefore, be distinguished from one another if a sufficiently large number of spores are measured. The same author (20) later showed that there are also slight but constant differences between the spore forms of different physiologic forms within the race *tritici* of *P. graminis*.

Mains (24) noted a certain amount of variation in the size of the urediospores and teliospores of some races of *Puccinia rubigo-vera*. In some cases such variation is quite marked, and this fact accounts for the tendency to separate certain groups of races as species. Mains (24), however, studied spore sizes of most of the races of *P. rubigo-vera* and stated that many races with aecia on *Thalictrum* did not differ with respect to urediospore size from the races on *Impatiens*, but that the latter are somewhat outstanding, as a group, for small size of the teliospores. He showed further that cer-

tain races having aecia on a variety of hosts, do not differ to any appreciable extent in teliospore size, but that a few other races with different aecial hosts do show appreciable differences. In general it was concluded that there is too much intergradation of the races of this rust to permit their being distinguished morphologically with any degree of certainty.

In connection with the physiological studies of the cultures of *Puccinia rubigo-vera* used in the present investigation a study of the comparative morphology of the urediospores and teliospores also was made. Only spores produced in the greenhouse, on the most susceptible hosts, and under uniform favorable cultural conditions were used.

For this study of the comparative morphology of 8 cultures of *Puccinia rubigo-vera*, the urediospores, being so nearly globoid, were measured for diameter only. The teliospores, of course, were measured in two dimensions. The length of the teliospore was considered to be that distance from the distal end to the septum separating the spore from the pedicel; the widest part was assumed to represent the diameter of the spore, exclusive of the thickened apex, which is sometimes wider than any other portion. One hundred and fifty urediospores and the same number of teliospores of each rust culture were measured. These were selected at random from a mount in lactic acid, (to render the germ pores more distinct and also to swell the spores to normal size). Only unquestionably mature spores were measured, the measurements being made to the nearest micron.

The data obtained from these measurements are presented in tables 3 to 5. They show rather marked differences between some of the cultures, especially if one compares their mean measurements. Table 3 shows the results obtained from the measurements of the urediospores of the 8 cultures. The range in diameter for the species as a whole, as represented by

TABLE 3.—Frequencies of the diameters of urediospores of 8 cultures of *Puccinia rubigo-vera*^a

Culture	Classes according to spore diameter									Mean diameter
	15 μ	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	μ
1				2	2	40	47	57	2	21.07
8				2	1	38	58	42	9	19.90
11			6	72	51	21				18.58
13			2	25	30	60	24	9		19.70
22	2	16	83	41	13					17.21
14	7	16	74	48	5					17.18
18			1	14	19	92	21	3		19.84
21				42	40	62	2	2		18.94

^a One hundred and fifty spores of each culture were measured.

TABLE 4.—Frequencies of the diameters of the teliospores of 8 cultures of *Puccinia rubigo-vera*^a

Culture	Classes according to spore diameter														Mean diameter
	8 μ	9 μ	10 μ	11 μ	12 μ	13 μ	14 μ	15 μ	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	μ
1			1	2	12	16	39	34	15	15	2	10	4		14.94
8			1	0	9	8	44	36	23	20	2	5	2		14.98
11					2	5	28	53	25	13	13	8	3		15.62
13				4	22	18	45	27	20	7	3	2	1	1	13.26
22			3	5	33	28	38	23	7	7	2	3	1		13.77
14			3	2	26	24	45	25	13	7	2	2	1		14.01
18	12	19	36	33	31	13	5	1							10.77
21	14	15	50	26	25	14	4	2							10.67

^a One hundred and fifty spores of each culture were measured.

these 8 cultures is 15–23 μ ; yet only 2 of the cultures (14 and 22) produced any spores that fell within the 15 μ class and only 2 (1 and 8) had any within the 23 μ class, insofar as this could be determined by measuring 150 spores. The modes for urediospore diameter vary from 17 μ , for cultures 14 and 22, to 22 μ for culture 1. The data (Table 3) show a similar difference in the mean diameters of the urediospores of various cultures. Culture 14 had the smallest mean diameter of urediospores, 17.18 μ . Culture 22 is scarcely larger, with a mean diameter of 17.21 μ . There is no correlation here of small urediospore size and aecial host, for 14 goes to *Impatiens* and 22 to *Clematis virginiana*. Two other cultures (18 and 21), having *Impatiens* for aecial hosts, have fairly large urediospores, with mean diameters of 19.84 μ and 18.94 μ respectively. The culture having the largest urediospores is number 1 (*Thalictrum* spp. aecial host), with a mean diameter of 21.07 μ .

The teliospores of these 8 cultures also showed differences. In table 4 it will be noted that, for the species as a whole, the teliospores range in diameter from 8 to 21 μ , the extremes being poorly represented. Two cultures are outstanding for narrow teliospores, culture 18 with a mean diameter of 10.77 μ , and culture 21 with a mean diameter of 10.67 μ . The teliospores with the largest mean diameter were obtained in culture 11, which was outstanding for scant and tardy production of teliospores.

The lengths of the teliospores of these cultures ranged from 24 to 53 μ (Table 5). Culture 21 is outstanding for short teliospores, having a mean length of 32.80 μ . Culture 1 was the largest, with a mean of 39.42 μ .

These data show that there is not necessarily a correlation between relative sizes of urediospores and teliospores. For instance, culture 11 has the widest teliospores of the 8 cultures, but the mean diameter of its urediospores is comparatively quite small. Teliospores of culture 18 have a comparatively small mean diameter, but the urediospores of this same culture are fairly large. However, culture 1 has both the largest urediospores and the largest teliospores of the 8 cultures.

Morphologically, a few of the cultures showed some differences. Both 22 and 14 could be separated from the remainder of the cultures because of their small urediospores (Fig. 1), but not from each other. Culture 21 is separable from the others because it has the smallest teliospores (Fig. 2). Culture 18 is separable from the rest by its teliospores of distinctive shape (Fig. 2), which are mostly narrowly wedge-shape and, therefore, differ from the teliospores of the other cultures.

Cultural Experiments with *Puccinia tomipara*

Culture 20. On *Bromus ciliatus* and *Thalictrum dioicum*. Culture 20 originated from aecia collected on *Thalictrum dioicum* near Ann Arbor,

TABLE 5.—Frequencies of the teliospores of 8 cultures of *Puccinia rubigo-vera*

Culture	Classes according to spore length															Mean length μ
	24- 25 μ	26- 27 μ	28- 29 μ	30- 31 μ	32- 33 μ	34- 35 μ	36- 37 μ	38- 39 μ	40- 41 μ	42- 43 μ	44- 45 μ	46- 47 μ	48- 49 μ	50- 51 μ	52- 53 μ	
1		2	2	5	14	24	35	18	16	15	4	8	4	2	1	39.42
8			3	9	16	17	40	18	17	17	5	1	5	2		38.75
11			5	12	14	26	44	15	22	9	2	0	1			37.03
13	2	6	7	13	18	22	39	11	14	4	4	7	3			35.96
22		1	2	10	14	11	32	16	12	20	11	8	6	4	3	39.10
14			6	13	14	30	33	22	14	6	6	4	1	1		37.85
18	2	6	9	14	15	11	27	27	18	14	4	3				36.85
21	2	9	15	26	35	28	19	11	1	4						32.80

^a One hundred and fifty spores of each culture were measured.

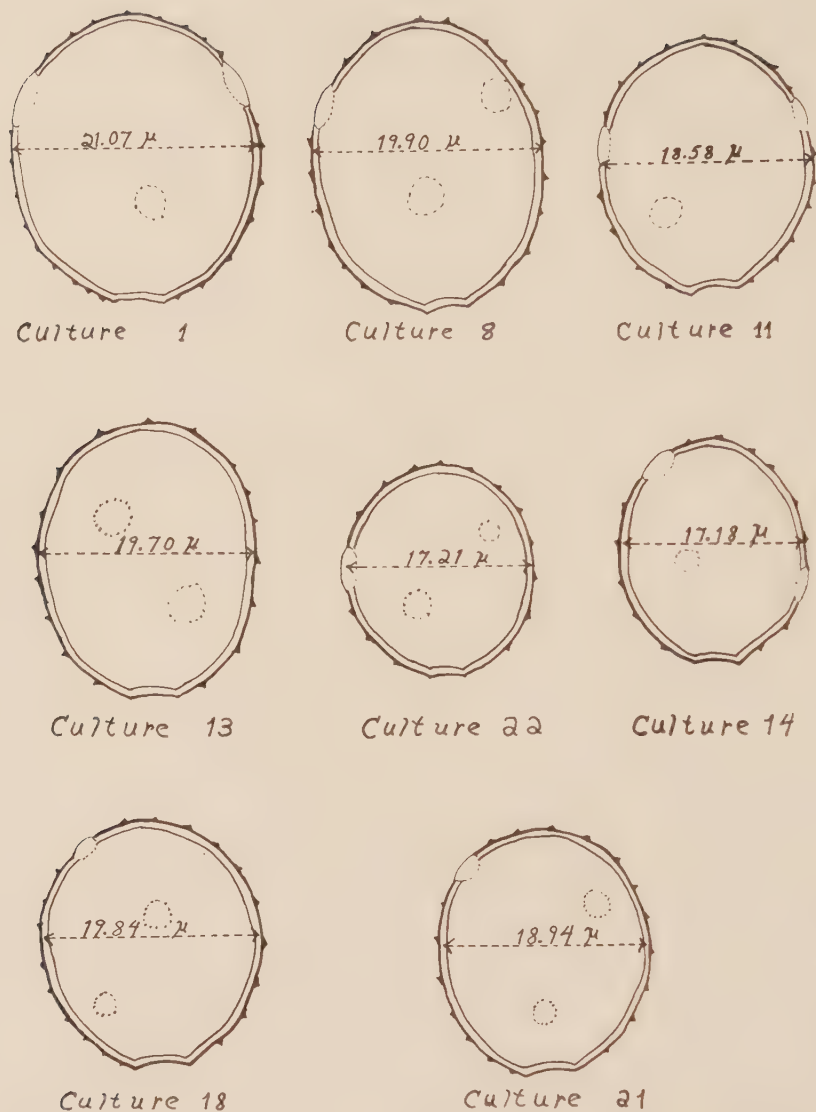


FIG. 1. Diagrams (median view) of representative teliospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean dimensions of the spores of each culture.

May 30, 1932. In close association with these aecia were the weathered remains of a species of *Bromus*, identified the following autumn as *B. ciliatus*. On the over-wintered leaves and sheaths from the previous season numerous telia of the leaf rust type were found. The teliospores were found to be multicellular and the rust was therefore, *Puccinia tomipara*.

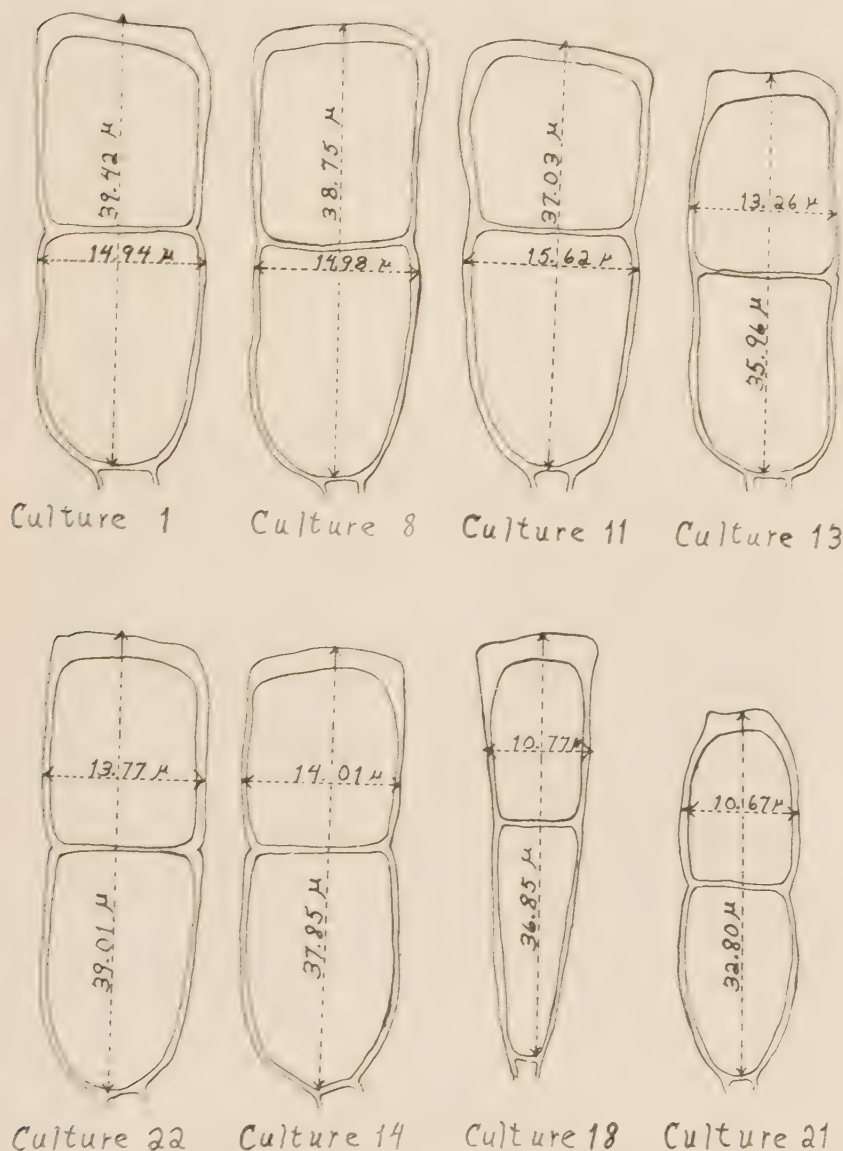


FIG. 2. Diagrams (median view) of representative urediospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean diameters of the spores of each culture.

The aecia were used to inoculate several species of *Bromus* in an attempt to find a favorable grass host. Uredia appeared on *B. purgans* only. This uredial culture was multiplied from time to time throughout the summer. Early in July telia began to appear; these continued to appear

in gradually increasing amounts, so that by the end of November only telia were produced. Examination showed that about 95 per cent of the spores of each telium were multicellular and many were muriform. The telia thus produced in the greenhouse from the original aecial collection, were overwintered in the usual manner. They were germinative even as early as March 8, 1933. A potted plant of *Thalictrum dasycarpum* was inoculated on March 9, resulting in pyenia on March 21 and open aecia on March 31. Seedlings of *B. ciliatus* (1649) and *B. purgans* (1289) were inoculated from these aecia, resulting in a heavy infection on both grasses. The culture was propagated in the usual manner. On May 17, the first telia were collected. Ninety to 95 per cent of the spores of these telia were multicellular, many of them consisting of 6 to 8 cells (Fig. 3). The infection of *T. dioicum* and



FIG. 3. Vertical section through a telium of *Puccinia tomipara* showing the multicellular structure of the teliospores. $\times 600$. (Photograph by E. B. Mains.)

T. dasycarpum was repeated several times. Grass inoculations with aecia collected in the field on *T. dioicum* and *T. dasycarpum* have shown that aecia of *Puccinia tomipara* and *P. rubigo-vera* (see culture 13) may occur side by side on leaves of the same plant and, no doubt, on the same leaf.

Other species of *Thalictrum* were tested for susceptibility to *Puccinia tomipara*. Inoculations of *T. fendleri*, *T. flavum*, and *T. glaucum* resulted in the production of pyenia and aecia on each.

The host range of *Puccinia tomipara* within the genus *Bromus* was studied in connection with a similar study of the *Bromus* culture (Culture

23) of *P. rubigo-vera*. The writer has observed both species present on the same grass hosts in the field, sometimes even intermixing on these grasses, as indicated above (Culture 23). *Puccinia tomipara* has been found in the field on *Bromus ciliatus* and *B. altissimus*.

Inoculations of a series of *Bromus* species with *Puccinia tomipara* have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus altissimus* (1795), *B. ciliatus* (1649), *B. purgans* (1289).

MODERATELY SUSCEPTIBLE: *Bromus rigidus* (1384, 1452), *B. rubens* (709, 1502).

DISTINCTLY RESISTANT: *Bromus adoensis* (1441, 1331, 815), *B. arduensis* (1332), *B. arvensis* (708, 1297), *B. brizaeformis* (1442), *B. commutatus* (716, 771, 1349, 1449, 1298), *B. hordeaceus* (719, 1508), *B. intermedius* Gus. (1285), *B. japonicus* (1347, 1551), *B. kalmii* (1541), *B. lanuginosus* (1550), *B. macrostachys* (797, 714, 705, 1446), *B. madritensis* (713, 1362, 1385, 1504, 1447), *B. maximus* (1427), *B. racemosus* L. (711), *B. rigidus* (1481, 1501, 698, 1505), *B. rigidus gussonii* (1506, 1333), *B. secalinus* L. (707), *B. sterilis* (1503), *B. squarrosus* (1450), *B. tectorum* (1547, 768, 1545, 712), *B. uniloides* (1507, 1451, 718, 750).

The specialization of this rust to such a few species of *Bromus* is interesting. Of 26 species of *Bromus* inoculated, only 3 closely related species are favorable hosts. Another fact to be noted is the lack of marked reactional differences between different collections within grass species. Only one species (*B. rigidus*) evinced any such differences.

DISCUSSION

It is shown that the multicellular character of the teliospores of *Puccinia tomipara* is constant. No reduction in the percentage of multicellular spores produced was observed while the rust was carried in culture from aecia to telia, back to aecia and then to telia again. Approximately 95 per cent of the teliospores of the telia ultimately produced from the second generation of aecia were multicellular. It is concluded, therefore, that this rust deserves specific rank.

Nine cultures of *Puccinia rubigo-vera* on wild grasses have been shown, in the experimental data presented, to be physiologically distinct. The distinctions have been determined on the basis of the infection type of 8 of the cultures on seedlings of 46 collections of wild grasses of the genera *Agropyron*, *Elymus*, *Hordeum*, and *Hystrix*, and of one (Culture 23) on species of *Bromus*. The specialization of the cultures to their aecial hosts serves further to distinguish some of the cultures. Two cultures (22 and 23) produce aecia on *Clematis virginiana*; three (14, 18, and 21) on *Impatiens biflora*, one (1) on exotic species of *Thalictrum* and one (13) on two native

species of *Thalictrum*; the aecial hosts for two (8 and 11) were not determined.

According to the concept employed by some investigators (Eriksson (8), Klebahn (17), Arthur and Fromme (4), Arthur (5), and others who have emphasized specialization to aecial host), a few of these cultures could be considered separate species. Culture 14, with aecia on *Impatiens biflora*, comes within the concept of *Dicaeoma impatientis* (Schw.) Arthur, (*Puccinia impatientis* (Schw.) Arth.) as given by Arthur and Fromme in North American Flora (4). The other two cultures (18 and 21), bearing aecia on *Impatiens* have urediospores somewhat larger than those described for that species. However, all three of these cultures having *Impatiens* for the aecial host conform well to the variety *P. rubigo-vera impatientis* (Arth.) Mains, as delimited by Arthur (5). Cultures 1 and 22, with aecia on *Thalictrum* and *Clematis*, respectively, agree with *D. clematidis* (DC.) Arth. as delimited by Arthur and Fromme (4) in North American Flora, except that the urediospores of culture 22 are too small. Culture 13, with aecia on *Thalictrum*, also might be placed in *D. Clematidis*, except in this case the teliospores are too small. This would indicate insufficient correlation between spore morphology of the leaf rust of the wild grasses and specialization to aecial hosts for a logical separation of species within the group, thus conforming to the conclusions of Mains (24), who recognized the whole group as an aggregate species, *P. rubigo-vera*, and erected 56 physiologic races, based on culture work of his own and that of previous investigators. Recently, Arthur (5) has recognized 6 varieties within the aggregate species, *P. rubigo-vera*, based chiefly on aecial- and telial-host restriction. Four of these, viz., *P. rubigo-vera tritici* (Eriks. and Henn.) Carl., *P. rubigo-vera secalis* (Eriks.) Carl., *P. rubigo-vera apocrypta* (Ellis and Tr.) Arth., and *P. rubigo-vera impatientis* (Arth.) Mains, are reasonably distinct because the aecia of each are restricted to a different family. In one case (the variety *impatientis*) this distinction is aided by the comparatively small size of the teliospores. The other two varieties, *P. rubigo-vera Agropyri*⁴ (Eriks.) Arth., and *P. rubigo-vera agropyrina* (Eriks.) Arth. have aecia on hosts of the same family (Ranunculaceae) and have essentially similar grass hosts. They are not morphologically distinct. It would be difficult to assign any of the 3 cultures of this study (nos. 1, 22, 13) having aecia on ranunculaceous hosts to one or the other of these two varieties.

Attempts to identify the 9 cultures of leaf rust of wild grasses with the 56 races of *Puccinia rubigo-vera* described by Mains (24) were unsuccessful.

⁴ Arthur (5) cites this as "*Puccinia rubigo-vera Agropyri* (Erikss.) n. comb. (*P. agropyri* E. and E.)." Since this varietal name apparently applies to the *Puccinia Agropyri* of Ellis and Everhardt rather than to Eriksson's *P. dispersa Agropyri* (*P. agropyrina*) the variety, if maintained, should be designated as *Puccinia rubigo-vera Agropyri* (E. and E.) Arth.

ful. Culture 1, common in Michigan on quack grass, *Agropyron repens*, might be *P. rubigo-vera* sp. f. *persistens* (Plowright) Mains. It, however, differs sharply from that race in that infection was obtained (reaction type 3-4) on strains of *Hordeum jubatum*, *H. pusillum* and *A. tenerum*.

Culture 8 on *Agropyron repens* from Indiana is almost identical in its morphology with culture 1, and, like culture 1, is similar to the race *persistens*. Culture 8, however, differs from this race in its ability to infect strains of *Elymus canadensis*. If either culture 1 or 8 is to be interpreted as belonging to race *persistens*, then the host range of that race must be extended and these 2 cultures interpreted as physiologic forms, differentiated by strains of a few species of grasses.

Culture 11 has been definitely shown to produce a type 3-4 reaction on *Agropyron tenerum*, *A. dasystachyum*, *Elymus canadensis*, *E. glaucus*, *E. robustus*, *E. striatus* var. *arkansanus*, *E. virginicus*, *E. virginicus* var. *glabriflorus*, *E. virginicus* var. *submuticus*, *Hordeum murinum* (1548 only) and *Hystrix patula*. In this combination of hosts the culture approximates *P. rubigo-vera* sp. f. *elymicola* Mains, but on the basis of the results reported by Mains (24) the race *elymicola* does not infect *A. tenerum* or *Hordeum murinum* and does infect *H. gussoneanum*. In a similar manner each of the 9 cultures of *P. rubigo-vera* used in these studies could be shown to differ in grass-host range from any of the 56 races of Mains (24).

Culture 11 might also be considered to be race *septentrionalis* Mains, which Mains (24) based on the studies made by Fraser (11) of a race of *Puccinia clematidis* having aecia on *Actea rubra*. However, definite determination of the race identity of culture 11 can not be made, because Fraser (11) did not inoculate *Agropyron tenerum*, *A. dasystachyum*, *Elymus glaucus*, *E. robustus*, *E. striatus* *arkansanus* and *Hordeum murinum*.

The fact that none of the 9 cultures can be assigned to any of the races of *Puccinia rubigo-vera* distinguished by previous investigators is due in part to the fact that many of the grasses used by the writer were represented by 2 to several reactionally different collections. The situation in the case of culture 22 may be cited to illustrate this point. This culture bears its aecia on Clematis, and might be expected to conform to one of the 9 races connected with Clematis and recognized by Mains (24). On the basis of the reaction of the grasses he inoculated, culture 22 most closely approximates his race *indianensis*, but apparently cannot be included in this race because *Agropyron caninum* (1466 only), *A. tenerum* (1659) and *Elymus glaucus* (1539 only) proved highly susceptible (resistant to race *indianensis*). Thus it is seen that if the writer's series of grasses had not included those particular strains of these 3 species, culture 22 might well have been considered as *P. rubigo-vera* sp. f. *indianensis* Mains. It might

be considered a physiologic form of this race, distinguished by the reactions of differential strains of *A. caninum*, *A. tenerum*, and *E. glaucus*.

Likewise, the use of more than one collection of the grass species has rendered a few of the cultures separable from the others where such separation would otherwise have been impossible or unsatisfactory. Thus, cultures 1 and 8 would have been considered identical had the series of grasses not chanced to include *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482), *E. virginicus* (1475), *Agropyron repens* (B.G. 13007) and *Hordeum gussoneanum* (1291). The other collections within these same grass species gave identical reactions to cultures 1 and 8. The situation in cultures 14 and 21 is similar, though perhaps less well-marked. These two cultures have the same aecial host (*Impatiens*) and would have been considered identical had not certain collections of grass species been included in the inoculations, viz. *E. canadensis* (843, 1305, 1366, 1537), *E. virginicus* (765), *A. caninum* (1466), *A. dasystachyum* (1656), *H. gussoneanum* (1421), *H. pusillum* (1650) and *Hystrix patula* (1477).

The manner in which the various strains within the wild grass species differ from each other is similar to the reactional differences that have been found in agronomic varieties of the cereals. The results of these investigations indicate that it would be possible for physiologic forms of the leaf rust of wild grasses to be distinguished one from the other by a standard set of strains or varieties within any one of several species of wild grasses.

The greater the number of strains or differentials used, the greater the possibilities of distinguishing divisions of the rust based on host specialization. If, as these studies indicate, there is in the races of leaf rust on wild grasses, a condition of physiologic specialization comparable to that in the races on the cereals (rye, wheat) almost innumerable forms might be differentiated if variously reacting strains of the wild-grass species are selected and employed.

In previous studies where physiologic forms have been distinguished by strains or agronomic varieties of a host species, only those belonging to one host species have been employed, as, for example, the use of agronomic varieties of wheat to distinguish physiologic forms of *Puccinia rubigo-vera tritici*. Apparently, no attempt has been made to determine the reaction of such physiologic forms on strains or varieties of other species of hosts. It is obvious that such a procedure would soon become very involved and hardly feasible. On the other hand, it is evident that if divisions of species of rusts are made on the basis of specialization to several species of hosts, intraspecific reactional differences should be taken into consideration in order that the results of various investigators may be comparable. As in the study of physiologic forms of the cereal rusts, a standard selected series of strains of the host species involved could be employed.

From the above discussion, the conclusion may be drawn that physiologic specialization in the leaf rust of wild grasses presents a situation quite comparable to that of the leaf rust of cereals and that interpretations of culture work should involve a consideration of the intraspecific reactional differences between collections of wild-grass species.

SUMMARY

Forty-six collections representing 18 species and varieties of wild grasses of the genera *Agropyron*, *Elymus*, *Hordeum*, and *Hystrix*, were studied with regard to their reaction to 8 cultures of *Puccinia rubigo-vera*.

The reactions of the wild-grass collections to these 8 cultures of leaf rust showed that the cultures are physiologically distinct.

Twenty-six species of *Bromus* were inoculated with 2 rusts of brome grass, 1 of which was a culture of *Puccinia rubigo-vera*, and the other, *Puccinia tomipara*.

Marked intraspecific reactional differences were noted for various collections of wild-grass species when inoculated with the various cultures of leaf rust.

In view of the fact that strains within wild-grass species may differ markedly from one another with regard to reaction to *Puccinia rubigo-vera*, it becomes evident that if the results of studies of host specialization of this rust are to be comparable, grasses of known genetic constitution should be employed.

Puccinia rubigo-vera on wild grasses presents a degree of physiologic specialization quite comparable to that characteristic of the races of cereal rusts.

Certain cultures of *Puccinia rubigo-vera* differed rather markedly from the others in mean dimensions of the spores. Cultures having the smallest urediospores do not necessarily have the smallest teliospores. Attempts to correlate spore size with host specialization were unsuccessful.

The multicellular character of the teliospores of *Puccinia tomipara* remained constant, through two generations. It is concluded, therefore, that this rust deserves specific rank.

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LITERATURE CITED

1. ARTHUR, J. C. Cultures of Uredineae in 1904. Jour. Mycol. 11: 50-67. 1905.
2. ————. Cultures of Uredineae in 1906. Jour. Mycol. 13: 189-205. 1907.
3. ————, ET AL. The plant rusts. 446 pp. John Wiley and Sons, New York, 1929.
4. ————. Uredinales. North American Flora 7: 83-969. 1907-1931.

5. ARTHUR, J. C. Manual of the Rusts in United States and Canada. 438 pp. Purdue Res. Foundation. Lafayette, Indiana. 1934.
6. DE CANDOLLE, A. Flora Française, famille des Champignons, 6: 10-115. 1815.
7. DIETEL, P. Reihe Uredinales. In Engler und Prantl, Nat. Pfl. 2te Aufl. 6: 24-98. 1928.
8. ERIKSSON, JAKOB. Nouvelles études sur la rouille brune des céréales. Ann. Sci. Nat. Bot. VIII, 9: 241-288, 1899.
9. ———, and ERNST HENNING. Die Hauptresultate einer neuen Untersuchung über die Getreideroste. Ztschr. f. Pflanzenkr. 4: 66-73, 140-142, 197-203, 257-262. 1894.
10. FRASER, W. P. Cultures of heteroecious rusts in 1918. Mycologia 11: 129-133. 1919.
11. ———. Cultures of *Puccinia Clematidis* (DC.) Lagerh. and *Puccinia impatientis* (Schw.) Arth. Mycologia 12: 292-295. 1920.
12. HASSEBRAUK, K. Gräserenfektion mit Getreiderosten. Arb. biol. Reichsanst. f. Land- u. Forstw. 20: 165-182. 1932.
13. JACKSON, H. S., and E. B. MAINS. Aecial stage of the orange leaf rust of wheat, *Puccinia triticina* Eriks. Jour. Agr. Res. 22: 151-172. 1921.
14. JOHNSTON, C. O. An aberrant physiologic form of *Puccinia triticina* Eriks. Phytopath. 20: 609-620. 1930.
15. ———, and E. B. MAINS. Studies on physiologic specialization in *Puccinia triticina*. U. S. Dept. Agr. Tech. Bul. 313. 1932.
16. ———, and L. E. MELCHERS. Greenhouse studies on the relation of age of wheat plants to infection by *Puccinia triticina*. Jour. Agr. Res. 38: 147-157. 1929.
17. KLEBAHN, H. Die wirtswechselnden Rostpilze. 447 pp. Gebrüder Borntraeger, Berlin. 1904.
18. LAGERHEIM, G. Sur un nouveau genre d'Uredinées. Jour. Bot. 3: 185-189. 1889.
19. LEVINE, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. Jour. Agr. Res. 24: 539-568. 1923.
20. ———. Biometrical studies on the variation of physiologic forms of *Puccinia graminis tritici* and the effects of ecological factors on the susceptibility of wheat varieties. Phytopath. 18: 7-123. 1928.
21. MAINS, E. B. Notes on greenhouse culture methods used in rust investigations. Proc. Ind. Acad. Sci. 33: 241-257. 1924.
22. ———. Rye resistant to leaf rust, stem rust, and powdery mildew. Jour. Agr. Res. 32: 201-221. 1926.
23. ———. Studies in rust resistance. Jour. Heredity 17: 313-325. 1926.
24. ———. Host specialization in the leaf rust of grasses, *Puccinia rubigo-vera*. Papers Mich. Acad. Sci. 17: 289-394. 1933.
25. ———, and H. S. JACKSON. Physiologic specialization in the leaf rust of wheat, *Puccinia triticina* Eriks. Phytopath. 16: 89-120. 1926.
26. FLOWRIGHT, C. B. A monograph of the British Uredineae and Ustilagineae. 347 pp. K. Paul, Trench and Co., London. 1889.
27. SCHEIBE, ARNOLD. Studien zum Weizenbraunrost *Puccinia triticina* Eriks. I. Methoden und Ergebnisse bei der Bestimmung seiner physiologischen Formen. Arb. Biol. Reichsanst. f. Land- und Forstw. 16: 575-608. 1928.
28. ———. Studien zum Weizenbraunrost, *Puccinia triticina* Erikss. III. Über die geographische Verbreitung der einzelnen physiologischen Formen und Formen-

- Kreise Deutschland und in seinem angrenzenden Gebieten. Arb. Biol. Reichsanst. f. Land- und Forstw. 18: 55-82. 1930.
29. STAKMAN, E. C., and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.
30. ———, and F. J. PIEMEISEL. Biologic forms of *Puccinia graminis* on cereals and grasses. Jour. Agr. Res. 10: 429-496. 1917.
31. TREBOUX, O. Infectionsversuche mit parasitischen Pilzen. II, III. Ann Mycol. 10: 303-306, 557-563. 1912.
32. TRELEASE, W. Preliminary list of Wisconsin parasitic fungi. Trans. Wis. Acad. Sci. 6: 106-144. 1885.
33. WATERHOUSE, W. L. Australian rust studies. I. Proc. Linn. Soc. N.S.W. 54: 615-680. 1929.
34. ———. On the production in Australia of two new physiologic forms of the leaf rust of wheat, *Puccinia triticina* Erikss. Proc. Linn. Soc. N.S.W. 57: 92-94 1932.
35. WESTENDORP, G. O. Notice sur quelques cryptogames inédites ou nouvelles pour la flore belge. Bul. Acad. Roy. Belgique, II, 18: 384-417. 1852.
36. WINTER, G. Uredineae. In Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich, und der Schweiz. Band I, Abt. 1. 1881.

